Towards proteogenomic assay development of critical illness: learning from injured sheep used as models of intensive care

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ABSTRACT

Sheep are invaluable production animals and not only contribute to the human food chain, but a source of natural wool, for diverse cultural use including fulfilling sacrificial requirements and their burgeoning contribution to translational research. Whilst there are many parallels in the pathophysiology of ailments or production needs and translational relevance between sheep and other livestock animals such as cattle and pigs, most research has only been performed with respect to the latter two species. A problem however arises in that not enough is known about the responses of sheep to a range of physiological and pathological events. This problem is confounded further by potential breed differences in response. It is therefore essential that a proteogenomic model is developed to accurately define sheep's response to stimuli, such as minimally invasive non-fatal experimental acute injury, before sheep can successfully be used as a model for other species. These observations taken together, led to the research question: *How can learning from sheep help in the development of proteogenomic assays?* It is believed that every injury is associated with characteristic changes in protein expression. This work aims to develop and optimise methods for understanding injury through proteogenomic approaches using mass spectrometry (MS). Understanding strategies for surviving acute injury down to molecular mechanisms involving protein expression could help in developing novel therapies for the benefit of sheep themselves and better production outcomes for sheep growers. Most crucial is that proteogenomic information could be used to improve gene annotations for the recently sequenced sheep genome.

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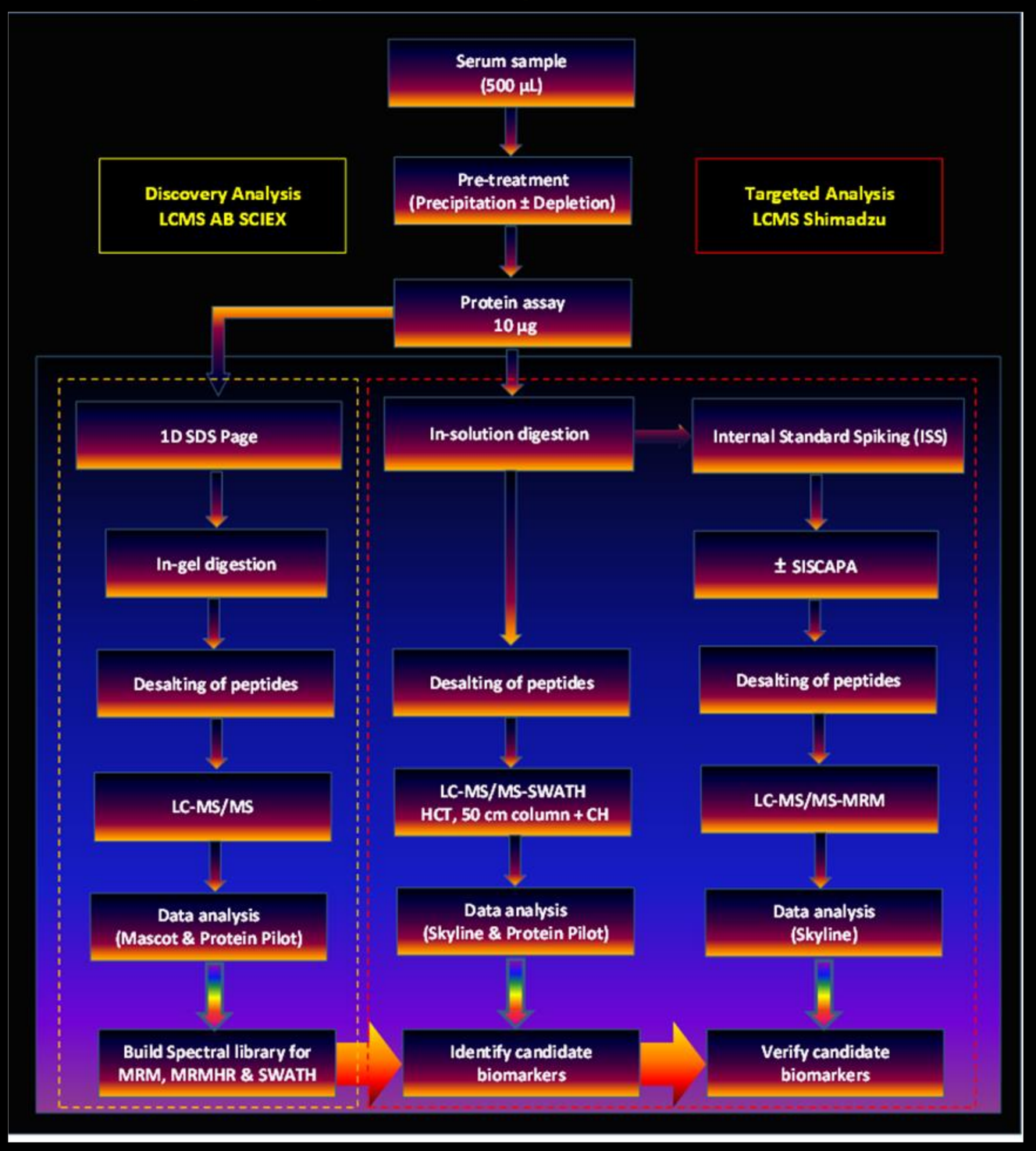
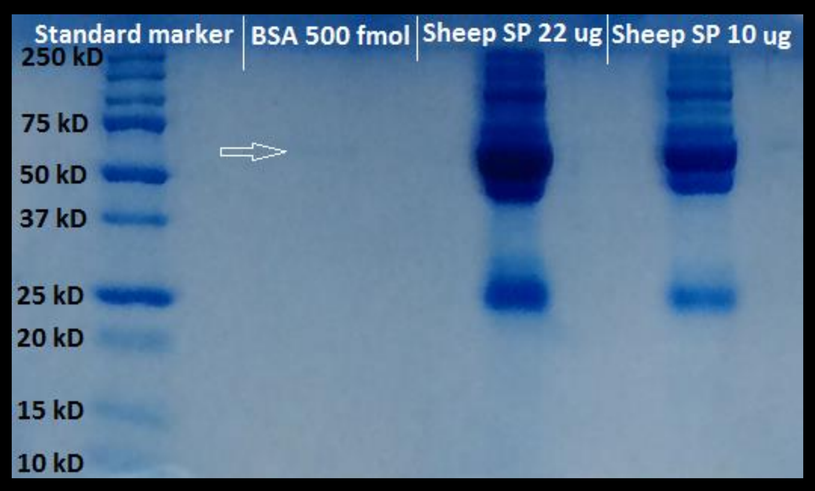
METHOD DEVELOPMENT

Proteogenomic characterisation of circulating acute phase markers and their bioassay development in sheep



Workflow for sheep protein biomarker discovery (left branch) and verification (right branch).

Key- SISCAPA: Stable isotope standard capture with anti-peptide antibodies; 1D: one dimensional; SDS-PAGE: sodium dodecyl sulphate-polyacrylamide gel electrophoresis; LC-MS/MS: liquid chromatography–tandem mass spectrometry; Protein Pilot: ProteinPilot™ proteomics software; HCT: high capacity trap column; CH: Column heater; MRM: Multiple reaction monitoring; MRMHR: High resolution multiple reaction monitoring; SWATH: Sequential window acquisition of all theoretical fragment-ion spectra



Results of discovery proteomics (Blue gel bands above) and targeted proteomic analysis to the right.---→